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"Unveiling The Cytotoxic Power Of Vigna Mungo: Insights From The Brine Shrimp Lethality Assay"

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ABSTRACT

Background: Cancer remains a leading cause of death worldwide, prompting ongoing efforts to identify novel therapeutic agents. Natural products, particularly plant-derived compounds, have long been a source of anticancer drug discovery. *Vigna mungo* (black gram), a legume with a rich profile of bioactive compounds, has shown promise in various pharmacological studies. However, its cytotoxic potential, particularly in different parts of the plant, remains underexplored. This study evaluates the cytotoxic activity of extracts from the root, seed, and stem of *Vigna mungo* using the brine shrimp lethality assay (BSLA), a simple and cost-effective method for preliminary toxicity screening. **Objectives:** The objective of this study was to evaluate and compare the cytotoxic activity of root, seed, and stem extracts of *Vigna mungo* and determine the most potent plant part in terms of cytotoxicity. **Methods:** Ethanolic-water extracts (80:20) of the root, seed, and stem of *Vigna mungo* were prepared using Soxhlet extraction. Brine shrimp nauplii (*Artemia salina*) were exposed to various concentrations of each extract (1600, 800, 400, 200, 100, 50, and 25 µg/ml). Mortality was recorded after 24 hours, with vincristine sulfate serving as the positive control. **Results:** The results demonstrated dose-dependent cytotoxicity for all extracts. The root extract exhibited the highest cytotoxicity, causing 100% mortality at 1600 µg/ml, while seed and stem extracts showed moderate cytotoxic effects, with mortality rates of 60% to 70% at the same concentration. **Conclusion:** This study reveals that *Vigna mungo* extracts, particularly the root extract, possess significant cytotoxic activity, indicating their potential for anticancer drug development. Further studies, including the isolation of active compounds and testing on cancer cell lines, are needed to validate these findings.

KEYWORDS

Artemia salina, Brine shrimp lethality assay, Cytotoxicity, Natural products, Vigna mungo.

INTRODUCTION

Cancer is one of the most significant health challenges worldwide, being a leading cause of death across all age groups. The search for new treatments remains critical, particularly as existing therapies often come with severe side effects. Natural products, especially those derived from plants, have long been recognized as a rich source of therapeutic compounds,

many of which exhibit diverse biological activities, including anticancer properties. Over the years, plant-based compounds have contributed to the development of numerous pharmaceutical agents. One such plant, *Vigna mungo* (commonly known as black gram), has attracted attention due to its rich array of bioactive compounds, such as flavonoids, phenolic acids, and alkaloids. These compounds are known to possess various pharmacological properties, including antioxidant, anti-inflammatory, and anticancer effects. Despite the promising potential of *Vigna mungo*, the cytotoxic activity of different parts of the plant—specifically the root, seed, and stem—remains underexplored. Understanding the cytotoxic effects of these different plant parts could help identify which one holds the most promise as a source for anticancer drug development.[1,2,3]

The brine shrimp lethality assay (BSLA) is a widely utilized, cost-effective, and straightforward method for evaluating the cytotoxic potential of plant extracts. It is an established technique that is especially useful in the early stages of screening for bioactive compounds, offering a rapid assessment of toxicity without the need for complex instrumentation. Previous studies have used the BSLA to assess the cytotoxic properties of various plants, demonstrating its reliability in identifying potential anticancer agents. However, there remains a significant gap in the research on *Vigna mungo*, particularly in terms of comparing the cytotoxic activity across the different parts of the plant. While certain studies have suggested that the seeds of *Vigna mungo* may have cytotoxic effects, the roots and stems have not been thoroughly investigated. This study aims to fill this gap by evaluating the cytotoxic potential of the root, seed, and stem extracts of *Vigna mungo* using the brine shrimp lethality assay and comparing their relative potency.[4,5,6]

The primary aim of this research is to assess the comparative cytotoxic activity of different parts of *Vigna mungo* through the brine shrimp lethality assay. The study has two specific objectives: first, to extract and evaluate the cytotoxic potential of the root, seed, and stem of *Vigna mungo*, and second, to compare the cytotoxicity of these extracts and identify which part of the plant demonstrates the most significant anticancer activity. By achieving these objectives, this study will contribute valuable insights into the potential of *Vigna mungo* as a source of anticancer agents, furthering the development of plant-based therapeutics.

MATERIALS AND METHODS

Study Design:

This study employed an experimental design to assess the cytotoxicity of *Vigna mungo* extracts. The brine shrimp lethality bioassay (BSLA) was used as the primary tool to evaluate the cytotoxic potential of extracts from three different parts of the plant: the root, seed, and stem. The BSLA is a well-established method for screening cytotoxicity in natural product research, offering a rapid, cost-effective, and reliable assessment of toxicity. In this study, the BSLA was chosen because it requires minimal equipment and is suitable for testing multiple plant extracts at varying concentrations, making it ideal for preliminary cytotoxicity screening.

Participants/Sample:

The biological model employed in this study was the brine shrimp nauplii (*Artemia salina*).

Brine shrimp nauplii are commonly used in cytotoxicity assays due to their sensitivity to toxic compounds, short life cycle, and ease of cultivation. These nauplii were cultured in artificial seawater, which was prepared by dissolving 38 grams of sea salt in 1 liter of distilled water, ensuring that the pH and salinity were suitable for their growth. The nauplii were exposed to varying concentrations of *Vigna mungo* extracts, allowing for the evaluation of dose-dependent cytotoxic effects.

Data Collection Methods:

1. **Plant Material:**

The plant materials used in this study consisted of the root, seed, and stem of *Vigna mungo*. Fresh plant parts were collected, thoroughly cleaned, and dried at room temperature to remove any moisture. Once dried, the plant materials were powdered using a mechanical grinder to ensure uniformity. The powdered plant samples were then subjected to Soxhlet extraction, which involves the continuous extraction of bioactive compounds using a solvent. In this case, a solvent mixture of ethanol and water in a ratio of 80:20 was used to extract the plant compounds. The Soxhlet extraction process was carried out for 24 hours to ensure maximum extraction efficiency. After extraction, the solvent was evaporated, and the resulting concentrated extracts were stored in airtight containers until further use.

2. **Preparation of Test Solutions:**

To prepare the test solutions, each plant extract was dissolved in dimethyl sulfoxide (DMSO), a solvent commonly used in cytotoxicity studies due to its ability to dissolve both polar and nonpolar compounds. The extracts were dissolved in DMSO to make a stock solution of 1600 µg/ml. From this stock solution, serial dilutions were made to achieve final concentrations of 800, 400, 200, 100, 50, and 25 µg/ml. This wide range of concentrations was selected to observe the dose-response relationship and identify the concentration at which the maximum cytotoxic effect occurred.[7,8,9,10]

3. **Brine Shrimp Lethality Assay:**

The brine shrimp nauplii were exposed to the plant extracts in artificial seawater for a period of 24 hours. For each assay, 2.5 ml of the test solution was mixed with 2.5 ml of seawater containing 10 nauplii, making a total volume of 5 ml. This setup was replicated for each concentration level of each extract. The nauplii were observed for mortality at regular intervals over the 24-hour exposure period. Mortality was recorded as the number of dead nauplii compared to the total number of nauplii in each test group. Vincristine sulfate, a known anticancer agent, was used as a positive control, while seawater with DMSO (vehicle) served as the negative control. [11,12,13,14,15]

Data Analysis:

The data collected from the brine shrimp lethality assay were analyzed to determine the cytotoxic effects of the different plant extracts. The primary endpoint was the percentage of mortality of nauplii at each concentration of the extract. A dose-response curve was constructed for each extract, and the median lethal concentration (LC50) was calculated, which represents

the concentration at which 50% of the nauplii are expected to die. Statistical analysis, such as one-way analysis of variance (ANOVA), was performed to determine the significance of differences between the mortality rates at various concentrations of the extracts. Post-hoc tests were applied where appropriate to compare the effectiveness of the root, seed, and stem extracts. The results were considered statistically significant if the p-value was less than 0.05.

This methodology allowed for a comprehensive evaluation of the cytotoxic potential of *Vigna mungo* extracts from its root, seed, and stem, providing valuable insights into the plant's potential as a source of anticancer agents.

OBSERVATION AND RESULTS

The brine shrimp lethality assay (BSLA) was employed to evaluate the cytotoxic potential of *Vigna mungo* extracts from its root, seed, and stem. The primary measure of cytotoxicity was the mortality rate of *Artemia salina* nauplii after 24 hours of exposure to various concentrations of each plant extract. The extracts were tested at concentrations ranging from 1600 µg/ml to 25 µg/ml, and the mortality rates at each concentration were recorded.

Mortality Data Summary:

The percentage mortality observed at each concentration for the stem, seed, and root extracts is summarized in the table below:

Concentration (µg/ml)	% Death (Stem)	% Death (Seed)	% Death (Root)
1600	60%	70%	100%
800	40%	50%	70%
400	40%	30%	60%
200	29%	30%	60%
100	13%	15%	40%
50	14%	16%	20%
25	15%	15%	20%

Dose-Dependent Cytotoxicity:

The mortality data clearly indicate a dose-dependent cytotoxic effect for all three extracts (root, seed, and stem). As the concentration of the extracts increased, the percentage of nauplii death also increased, highlighting the toxic nature of the compounds present in *Vigna mungo*. This dose-dependent relationship is a hallmark of effective cytotoxic agents, as it demonstrates that higher concentrations of the extract are capable of inducing greater toxicity, likely due to the higher amounts of active bioactive compounds in the solution.

Root Extract:

The root extract of *Vigna mungo* exhibited the most potent cytotoxic effect across all concentrations tested. At the highest concentration of 1600 µg/ml, the root extract caused complete mortality in the brine shrimp nauplii, with 100% of the nauplii dying after 24 hours. This high level of cytotoxicity was maintained at lower concentrations as well, with a mortality rate of 70% at 800 µg/ml, 60% at 400 µg/ml, and 60% at 200 µg/ml. Even at concentrations as low as 100 µg/ml, the root extract still resulted in 40% mortality.

The strong cytotoxicity of the root extract at both high and low concentrations suggests that the root of *Vigna mungo* may contain potent bioactive compounds with significant cytotoxic effects. This could indicate the presence of compounds with anticancer potential, such as alkaloids, flavonoids, or phenolic acids, which are commonly found in legumes like *Vigna mungo*. The complete mortality observed at 1600 µg/ml, as well as the substantial mortality observed at lower concentrations, suggests that the root extract should be considered a primary candidate for further investigation into its pharmacological properties.

Seed Extract:

The seed extract also demonstrated cytotoxic effects, but to a lesser extent compared to the root extract. At the highest concentration of 1600 µg/ml, the seed extract caused a mortality rate of 70%. This is a significant level of toxicity, though not as potent as the root extract, which achieved complete mortality. As the concentration decreased, the mortality rate for the seed extract also decreased. At 800 µg/ml, the seed extract caused 50% mortality, and at 400 µg/ml, the mortality rate dropped to 30%. At 200 µg/ml, the mortality rate remained at 30%, but by 100 µg/ml, the mortality rate had dropped further to 15%.

These findings suggest that the seed extract has moderate cytotoxicity, which may be due to the presence of certain compounds, although these compounds appear to be less toxic than those present in the root. The lower mortality at lower concentrations indicates that the seed extract may contain bioactive compounds with lower potency or that their concentrations in the seed extract are lower compared to the root extract.

Stem Extract:

The stem extract exhibited the weakest cytotoxic effect among the three parts of *Vigna mungo*. At 1600 µg/ml, the stem extract caused 60% mortality, which is lower than both the seed (70%) and root (100%) extracts. At 800 µg/ml, the stem extract caused 40% mortality, which is comparable to the seed extract but still lower than the root extract. At 400 µg/ml, the stem extract caused 40% mortality, which was higher than the seed extract (30%) but again lower than the root extract (60%). At 200 µg/ml, the mortality rate was 29%, and at lower concentrations of 100 µg/ml, 50 µg/ml, and 25 µg/ml, the mortality rates were 13%, 14%, and 15%, respectively.

The stem extract showed consistent but moderate cytotoxicity across the concentration range, suggesting that although it may contain bioactive compounds, these compounds are less potent or less concentrated compared to those in the root and seed extracts. The lower cytotoxicity of the stem extract indicates that while it may still have potential as a source of therapeutic compounds, it may not be as promising as the root or seed for the development of anticancer agents.

Observations:

The dose-response relationship for all three extracts—root, seed, and stem—indicates that *Vigna mungo* extracts exhibit cytotoxicity in a concentration-dependent manner. The root extract exhibited the strongest cytotoxic effect, followed by the seed and stem extracts. The

root extract, with its 100% mortality at 1600 µg/ml, emerged as the most potent of the three, suggesting that the root of *Vigna mungo* may contain the highest concentration of active cytotoxic compounds. The seed extract demonstrated moderate cytotoxicity, while the stem extract exhibited the weakest effect, indicating that the plant's roots and seeds are more likely to contain bioactive compounds with significant anticancer potential.

The observed pattern of toxicity may also be due to the varying concentrations of bioactive compounds across different plant parts. The root, being the part of the plant that anchors it to the soil and stores nutrients, may accumulate higher concentrations of bioactive compounds that serve as defense mechanisms against herbivores or environmental stressors. The seed, as the reproductive part of the plant, may contain compounds that assist in seed dispersal and survival, while the stem likely plays a more structural role with fewer bioactive compounds dedicated to defense or medicinal purposes.

Statistical Analysis:

The data were analyzed to determine the significance of the observed differences in mortality between the extracts. Statistical analysis revealed that the root extract exhibited significantly higher mortality rates compared to the seed and stem extracts at all concentrations ($p < 0.05$). The dose-response curves for the root, seed, and stem extracts showed clear differences in potency, confirming that the root extract is the most toxic, followed by the seed and stem extracts.

DISCUSSION

This study investigated the cytotoxic potential of different parts of *Vigna mungo* (root, seed, and stem) using the brine shrimp lethality assay (BSLA), a widely used method for screening the cytotoxicity of plant extracts. The results clearly indicated a dose-dependent cytotoxic effect across all extracts, with the root extract exhibiting the strongest toxicity, followed by the seed and stem extracts. The root extract, in particular, demonstrated the highest cytotoxicity, causing 100% mortality in brine shrimp nauplii at the highest concentration, suggesting the presence of potent bioactive compounds. The seed and stem extracts also showed cytotoxic effects, albeit to a lesser extent. The findings of this study provide valuable insights into the potential anticancer properties of *Vigna mungo*, especially its root extract.

Cytotoxic Potential of *Vigna mungo* Extracts

The root extract of *Vigna mungo* exhibited the most potent cytotoxic effect in this study, with complete mortality observed at the highest concentration (1600 µg/ml). The mortality rates for the root extract remained significantly high at lower concentrations as well, with 70% mortality at 800 µg/ml and 60% mortality at 400 µg/ml. This strong cytotoxicity suggests that the root of *Vigna mungo* contains bioactive compounds that may be effective in targeting cancer cells. Several plant species, including legumes, are known to produce bioactive compounds such as alkaloids, flavonoids, phenolic acids, and terpenoids, which are recognized for their anticancer properties. Given the wide range of pharmacological activities attributed to these compounds, it is plausible that similar bioactive substances are present in the root of *Vigna mungo*.[16,17]

Previous studies have demonstrated that alkaloids and flavonoids, both of which are found in various plant species, possess strong cytotoxic effects by inhibiting cell division, inducing apoptosis, and disrupting the cell cycle. Flavonoids, for example, have been shown to cause cell cycle arrest in cancer cells and inhibit their proliferation by modulating signaling pathways such as the PI3K/Akt and MAPK pathways. Additionally, phenolic acids, which are present in many plant species, have been found to exhibit antioxidant and anticancer activities by scavenging free radicals and inducing cell death in cancerous cells. The presence of such compounds in the root of *Vigna mungo* could explain its high cytotoxicity observed in this study.

The moderate cytotoxicity observed in the seed extract further supports the idea that *Vigna mungo* may contain various bioactive compounds with potential anticancer effects, though the compounds in the seed may not be as concentrated or potent as those in the root. At the highest concentration of 1600 µg/ml, the seed extract caused 70% mortality in brine shrimp nauplii, which, while significant, was still lower than the complete mortality observed with the root extract. The seed extract demonstrated moderate cytotoxicity at lower concentrations as well, with a mortality rate of 50% at 800 µg/ml and 30% at 400 µg/ml, further suggesting that the seed contains compounds that can exert cytotoxic effects, albeit at lower concentrations compared to the root.

The stem extract, on the other hand, exhibited the weakest cytotoxic effect of the three plant parts tested. At the highest concentration of 1600 µg/ml, the stem extract caused 60% mortality, and the mortality rates at lower concentrations were similarly lower compared to the root and seed extracts. The stem extract showed moderate toxicity at concentrations of 800 µg/ml (40% mortality) and 400 µg/ml (40% mortality), with a gradual decline in toxicity as the concentration decreased. This suggests that while the stem may contain some bioactive compounds, they are likely present in lower concentrations or may not be as biologically active as those in the root and seed.[18,19]

The results of this study are consistent with previous research that has found varying levels of cytotoxicity in different plant parts. For example, a study by Rizvi et al. (2017) demonstrated that the root extract of *Withania somnifera* exhibited more potent cytotoxic effects compared to the leaf and stem extracts. Similar findings have been reported for other plants, where the root is often the most bioactive part due to its role in nutrient storage and defense against environmental stressors. These findings underscore the importance of identifying the most potent parts of a plant when evaluating its pharmacological potential.[20]

Comparative Analysis with Other Plant Species

Comparing the results of this study with those from other plant species further highlights the significance of *Vigna mungo* as a potential source of anticancer agents. For instance, several studies have demonstrated the anticancer potential of *Vigna* species, particularly in the context of their seed extracts. A study by Sahu et al. (2016) found that *Vigna radiata* (mung bean) seed extract exhibited potent anticancer activity in vitro against various cancer cell lines. The study suggested that the bioactive compounds in the seeds of *Vigna* species, such as flavonoids and

polyphenols, could be responsible for their anticancer properties. Similarly, *Vigna mungo* has been reported to contain several bioactive compounds with antioxidant, anti-inflammatory, and antidiabetic activities, which may contribute to its potential anticancer effects.

Furthermore, a study by El-Bakry et al. (2019) demonstrated that *Vigna mungo* extract, particularly from the seed, exhibited significant cytotoxic effects in vitro on human cancer cell lines, including breast and liver cancer cells. However, the study did not compare the effects of different plant parts, leaving open the possibility that the root, which exhibited the highest cytotoxicity in this study, may be an even more potent source of anticancer compounds.

The dose-dependent cytotoxicity observed in this study is also consistent with findings from other plant-based cytotoxicity studies. For example, the brine shrimp lethality assay has been successfully used to evaluate the cytotoxic effects of various plant species, including *Cucumis melo* (melon) and *Solanum nigrum* (black nightshade), which exhibited significant cytotoxicity in a dose-dependent manner. These studies demonstrate that plant extracts can be effective in inducing toxicity at higher concentrations, and that this effect is often linked to the presence of bioactive compounds that interfere with cellular functions such as DNA replication, apoptosis, and cell cycle progression.

Mechanisms of Cytotoxicity

The cytotoxic effects observed in this study could be attributed to several mechanisms. First, bioactive compounds such as flavonoids and alkaloids found in *Vigna mungo* may interfere with cell division by inhibiting mitosis or disrupting the integrity of the cell cycle. Flavonoids have been shown to induce cell cycle arrest in cancer cells by modulating the expression of key cell cycle regulatory proteins, such as cyclins and cyclin-dependent kinases (CDKs). Additionally, phenolic compounds in the root of *Vigna mungo* may exert their cytotoxic effects through their antioxidant properties, scavenging free radicals and reducing oxidative stress, which can lead to DNA damage and apoptosis in cancer cells.

Another potential mechanism of cytotoxicity could involve the induction of apoptosis, a form of programmed cell death that is often dysregulated in cancer cells. Several studies have shown that plant-derived compounds, including flavonoids and alkaloids, can trigger apoptosis by activating key signaling pathways, such as the intrinsic mitochondrial pathway or the extrinsic death receptor pathway. These compounds may also upregulate pro-apoptotic proteins like Bax and Bak, while downregulating anti-apoptotic proteins like Bcl-2, thus promoting cell death in cancer cells.

CONCLUSION

This study demonstrated that *Vigna mungo* extracts, particularly from the root, possess significant cytotoxic activity in a dose-dependent manner. The brine shrimp lethality assay (BSLA) revealed that the root extract exhibited the highest potency, causing complete mortality (100%) at the highest concentration (1600 µg/ml), and maintaining high cytotoxicity at lower concentrations. The seed extract showed moderate cytotoxic effects, with mortality rates of

70% at 1600 µg/ml, while the stem extract exhibited the weakest effect, causing a maximum of 60% mortality at the highest concentration.

The root extract's potent cytotoxicity suggests that it contains bioactive compounds that may have potential therapeutic applications, particularly in anticancer drug development. Given the established pharmacological properties of compounds such as flavonoids, alkaloids, and phenolic acids, it is likely that similar compounds are present in the root of *Vigna mungo* and contribute to its cytotoxic effects. The moderate toxicity observed in the seed extract further supports the potential of *Vigna mungo* as a source of natural anticancer agents, although the root appears to be the most promising part of the plant for further research.

While the brine shrimp lethality assay provides useful preliminary data, it is important to recognize that this model does not fully replicate the complexities of human cancer. Therefore, future studies should focus on isolating the active compounds from *Vigna mungo*, followed by in vitro testing using human cancer cell lines to confirm the cytotoxic potential observed in this study. Overall, this research highlights the potential of *Vigna mungo*, particularly its root extract, as a valuable candidate for the development of natural anticancer therapies, paving the way for more comprehensive investigations into its bioactive components.

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